

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NOTE TO FILE
BNF43
July 1, 1997

Subject: Virus-resistant squash line CZW3

Background

In a submission dated February 26, 1997, Seminis Vegetable Seeds, Inc., provided summary information to support their safety and nutritional assessment of their virus-resistant squash line CZW3. A summary of the nutrient analyses for the new variety of squash and its non-transgenic progenitor was submitted by Seminis on May 6, 1997.

Intended Effect of the Genetic Modification

The CZW3 squash was derived from a yellow crookneck squash, line YS20. The CZW3 line contains the coat protein genes of three viruses, cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus 2 (WMV2). The only portions of the CMV, ZYMV and WMV2 genomes that were introduced into plants were the genes encoding the coat proteins. The gene for neomycin phosphotransferase II (nptII) was introduced for use as a selectable marker. A single copy of each gene integrated into the genome. With the *Agrobacterium tumefaciens*-mediated plant transformation system, usually only DNA contained within the T-DNA borders is transferred into the plant genome. Occasionally, however, DNA outside the T-DNA borders is transferred into the plant genome. Seminis reports that Southern blot analyses indicate that no DNA outside the T-DNA borders was transferred into the CZW3 squash genome.

Regulatory Considerations

The use of the CMV, ZYMV and WMV2 coat proteins as pesticidal substances and the use of the selectable marker, nptII, as a pesticidal inert ingredient in the development of the virus-resistant squash are under the regulatory purview of the Environmental Protection Agency (EPA). EPA regulates the use of the introduced genetic material encoding the viral coat proteins and the selectable marker (including associated sequences required for expression) as well as the expression products. Therefore, in the consultation, we did not address the safe use of the viral coat proteins as pesticides or the safe use of nptII in squash as a pesticidal inert ingredient. The main focus of the consultation with FDA was the compositional analysis of the transgenic squash as compared to the parental variety.

Compositional Analysis

Endogenous toxicants

Seminis noted that cucurbits, including squash, are known to produce very bitter alkaloids known as cucurbitacins. Seminis also pointed out that above normal levels of cucurbitacins

can be detectable by taste, and in the literature, a correlation between taste and detection via laboratory methods has been observed. Seminis referenced published literature which documented that the detection level by taste was as low as 1 part per billion (ppb) for cucurbitacin B and 10 ppb for cucurbitacin E glycoside (cucurbitacin E is the primary cucurbitacin in squash). According to the Merck Index, 1989, the oral LD₅₀ in mice for cucurbitacin E is 340 mg/kg. Seminis concluded that the detection level of cucurbitacin E by taste is 34,000 lower than the LD₅₀. Seminis noted that a standard test for the presence of cucurbitacin E in squash breeding programs involves the tasting of fruits to determine bitterness. According to Seminis, the non-transgenic progenitor, a yellow crookneck squash line YS20, and the transgenic line CZW3 were taste tested and both were non-bitter.

Concentration of Important Nutrients

Seminis conducted nutrient analyses on their transgenic squash and on the parental variety. The analysis consisted of a single assay of two composite samples of squash fruit harvested from a field trial (20 fruit of transgenic squash line CZW3 and 11 fruit of non-transgenic parental squash line YS20). Analyses were performed to determine content of protein, moisture, total fat, ash, dietary fiber, total carbohydrate, calories, sugar profile (fructose, glucose, galactose, sucrose, maltose, and lactose), total vitamin C, beta carotene, vitamin A from carotene, calcium, iron, and sodium. Based on these analyses, Seminis concluded that the concentrations of nutrients in the transformed squash were comparable to those in the non-transgenic parental variety and fell within the literature values for squash.

Conclusions

Seminis has concluded that the CZW3 squash they have developed is substantially equivalent, in terms of food safety and nutritional profile, to existing squash varieties with a history of safe use. At this time, based on Seminis's description of its data and analysis, the Agency considers Seminis's consultation on this product to be complete.

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